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to bulk assays, the smFRET data indicate that hippuristanol does not appear to inhibit RNA binding to eIF4AI/eIF4H complexes (Bordeleau et al., 2006). The reason for this discrepancy is not clear, but it may be due to the ability of eIF4H to bind RNA loops and stabilize eIF4AI on the RNA substrate. Because eIF4AI is an attractive therapeutic target for inhibiting translation initiation, it will be interesting to use this approach to determine if other small molecule inhibitors can be found that target other steps in the helicase cycle.

Overall, this study elegantly explains the mechanism of eIF4AI conformational changes during RNA unwinding using a single molecule approach. The role of additional physiologically relevant eIF4AI stimulating proteins including eIF4G, eIF4E, and eIF4B remains to be determined. Interestingly, an independent study just published elsewhere used a similar method to examine how eIF4G, eIF4B, and different RNAs modulate the conformational cycle of yeast eIF4AI (Harms et al., 2014). The addition of eIF4G and eIF4B in those experiments accelerates the cycling of eIF4AI conformations, which is consistent with the observations that eIF4G and eIF4B cooperatively activate human eIF4AI duplex unwinding in bulk assays (Özeş et al., 2011; Parsyan et al., 2011). It will now be important to determine the dynamics of eIF4AI helicase activity in combination with the small ribosomal subunit during scanning through structured 5′ UTRs. Although the general ATPase conformational cycle is likely to be universal among DEAD box proteins, it is desirable to use the approach described by Sun et al. (2014) to explore the relationship between the conformational cycle and duplex unwinding for different DEAD box proteins given their different accessory factor requirements.

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Life in the "Old Bag" Yet: Structure of Peptidoglycan L,D-Carboxypeptidases

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In this issue of *Structure*, Hoyland and colleagues describe the structure of a peptidoglycan L,D-carboxypeptidase in both substrate-bound and apoenzyme forms. These studies reveal the basis for enzyme specificity and assist greatly in a field where form and function overlap.

The cell wall polymer peptidoglycan, sometimes referred to as the sacculus or murein, is of paramount importance to the majority of bacteria. A cursory (but far from exhaustive) list implicates peptidoglycan in a wide variety of physiological roles; it protects against osmotic pressure, is a barrier to external assault, is a conduit for secretion systems and tether for secondary polymers and proteins, is a primary driver of cell shape, is a source of immunogenic material, and even functions as a "sponge" for nutrient capture. When coupled with the attractiveness of peptidoglycan biosynthesis as a target for the development of antimicrobials, this strong linkage to bacterial physiology results in peptidoglycan metabolism being a highly active field of study. Despite this, several cell wall-modifying activities remain undercharacterized. In particular, D,D-transpeptidases, which are responsible for 3,4 crosslinks, are relatively much better

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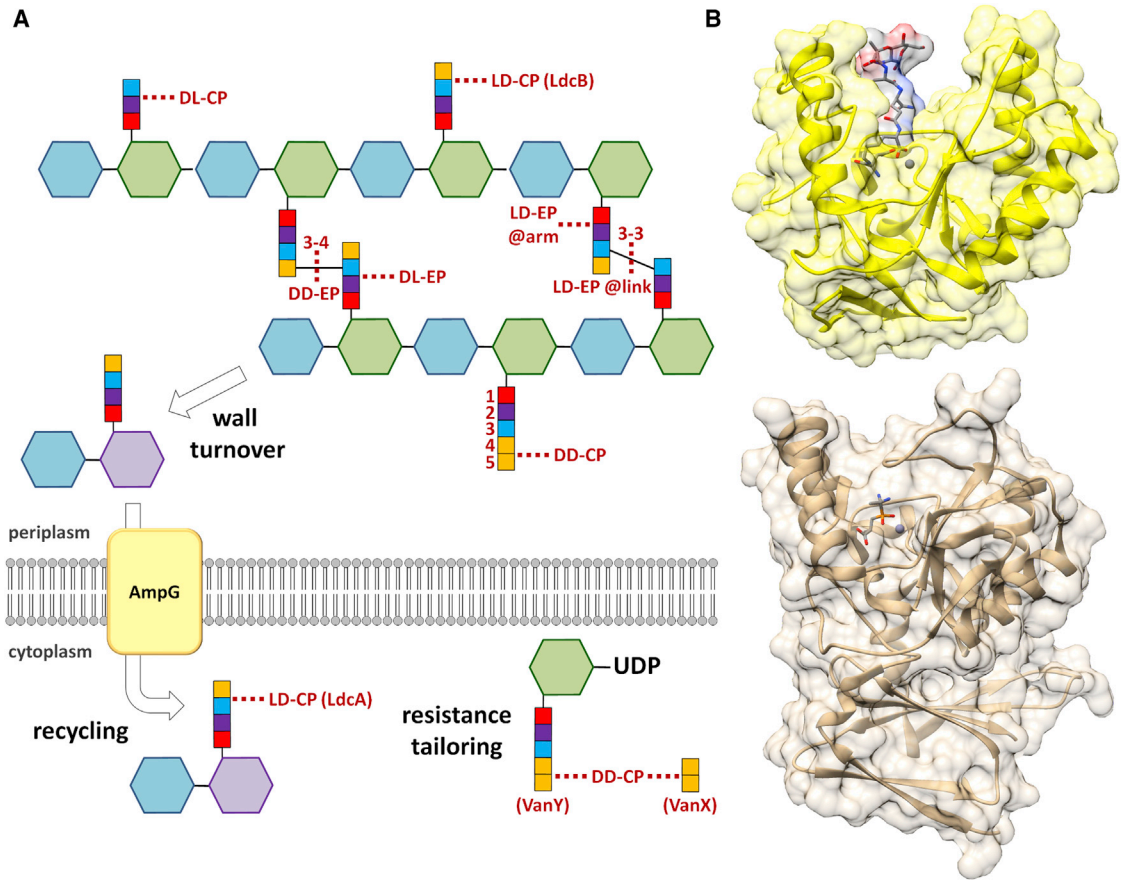

Figure 1. Peptidoglycan Metabolism by Carboxypeptidases and Endopeptidases

(A) Schematic of peptidoglycan and its metabolism by carboxypeptidases (CP, releasing a terminal amino acid) and endopeptidases (EP, cutting within a polypeptide); enzyme activity is represented by a red dashed line. The polymer comprises *N*-acetylmuramic acid (green hexagon) and *N*-acetyl glucosamine (blue hexagon) units, the former of which are substituted by a pentapeptide whose numbering and stereochemistry is noted (units 1–5, involved in 3-4 and 3-4 crosslinks, of varying D- and L-linkage). The mature wall polymer is acted upon by lytic enzymes, which generate 1,6-anhydro *N*-acetylmuramic acid (purple hexagon) fragments that are internalized by the membrane protein AmpG and subsequently recycled. Vancomycin-resistance involves removing the vancomycin target D-Ala-D-Ala present on the wall precursors (VanY on the UDP [uridine diphosphate]-linked sugar pentapeptide; VanX on the isolated dipeptide).

(B) Structure of the LdcB (RCSB accession 4OXD, top panel) and $VanXY_G$ (4MUQ, lower panel) LAS superfamily members in complex with their ligands (gray stick) and Zn cofactor (sphere representation).

understood than their L,D-transpeptidase counterparts responsible for 3,3 cross-links. This difference in understanding is partly due to the differential abundance and distribution of the two cross-linking types and partly due to the former being the target of β-lactams. This disparity is even more pronounced when carboxypeptidases (enzymes that trim mature peptidoglycan) are considered (Figure 1A).

In this issue of *Structure*, Hoyland et al. (2014) have determined the high-resolution structures of three peptidoglycan L,D-carboxypeptidases (L,D-CPs) from selected Gram-positive bacteria, one of which is bound to a near-mimic of its saccharide-linked tetrapeptide substrate. The peptidoglycan stem pentapeptide comprises both L- and D-amino acids, with a consensus sequence of L-Ala^1-D-Glu^2-meso-DAP(diaminopimelate)/L-Lys^3-D-Ala^4-D-Ala^5, and hence has a combination of L-D, D-L and D-D linkages. The pentapeptide can be trimmed down one amino acid at a time, such that the terminal amino acid to be removed by carboxypeptidase activity is successively in a D,D linkage (pentapeptide), L,D linkage (tetrapeptide), D,L linkage (tripeptide), and L,D linkage (dipeptide). An amidase is responsible for further removal of the L-Ala^1 from the muramic acid sugar. Historically, our understanding of CP activity has been limited to LdcA (*L,D*-carboxypeptidase *A*), a cytoplasmic enzyme responsible for recycling peptidoglycan fragments (Figure 1A). LdcA has been shown to adopt a sheet/barrel hybrid fold with a Ser-Glu-His catalytic triad (Korza and Bochtler, 2005). The first biochemical identification of the structurally unrelated periplasmic L,D-CPs, which instead act on the mature wall, came a year later with verification that disruption of a gene possessing 30% sequence identity to the VanY vancomycin-resistance carboxypeptidase resulted in a decreased incidence of tripeptides (Courtin et al., 2006). Importantly, this gene (named *dacB*, for *D-A*la *c*arboxypeptidase) contained a putative signal peptide, but the *dacB*-compromised strain did not show any obvious growth defects. A homolog of

dacB in *Streptococcus pneumoniae* (represented by one of the three structures solved by Hoyland et al., 2014) was implicated in cell shape and septation (Barendt et al., 2011), presumably because carboxypeptidase activity eliminates the fourth amino acid central to 3,4-crosslink formation.

Indeed, the hydrolytic activity of peptidoglycan endopeptidases/carboxypeptidases is intimately associated with various aspects of cell shape in several bacteria (for informative broad reviews see Wyckoff et al., 2012 and Frirdich and Gaynor, 2013). The vast complexity of the enzymes that catalyze these reactions can be appreciated when a third family of L,D-CPs, distinct from LdcA and those solved by Hoyland et al. (2014), is considered. These enzymes catalyze an identical carboxypeptidase reaction at the 3-4 linkage but display homology to L,D-transpeptidases responsible for formation of the 3-3 crossbridges; these CPs are found in the ε-proteobacteria and contribute to the helical morphology of the *Helicobacter pylori* and *Campylobacter jejuni* pathogens (Sycuro et al., 2013, Frirdich et al., 2014). It is currently impossible to assign and distinguish L,D-TP versus L,D-CP function via bioinformatics alone. Clearly, we have much yet still to learn! It is in this context that the work by Hoyland et al. (2014), wherein they reveal the determinants for substrate specificity and confirm activity using purified protein and sacculi, can further inform the field. From this, the authors suggest a name change from dacB to ldcB (*L*,*D*-carboxypeptidase *B*) to better describe the precise nature of these enzymes. The LdcB proteins are shown to adopt the LAS superfamily fold (Figure 1B), a Zn-dependent domain named after the *l*ysostaphin/D-*A*la-D-Ala-CP/sonic hedgehog family members. The importance of verifying specificity and understanding the overlapping form and functions of peptidoglycan-metabolizing enzymes is key here, because the LAS fold is used by LdcB, D,D-CPs (VanX, VanY, VanXY; Meziane-Cherif et al., 2014), L,D-EPs (CwlK and the endolysin Ply500; Korndörfer et al., 2008), and D,D-EPs (the morphology determinant MepA; Marcyjaniak et al., 2004). Broadly, it is yet to be established whether this multiplicity of roles has arisen via convergent or divergent evolution, although the Meziane-Cherif study comments on the particular evolution of altered specificity among the VanX, VanY, and VanXY members.

Comparing the structure of the substrate analog-bound *S. pneumoniae* LdcB to the apoenzyme structure reveals that the L,D-CPs likely employ an induced-fit mechanism, wherein E204 (which is responsible for stereochemical discrimination of substrate and also orientation of the potential nucleophillic water) alters conformation between the two states. This shift is driven by the reordering of a loop (residues 163–171) that otherwise occludes substrate entry to the active site cleft. Both the *S. pneumoniae* and *Bacillus subtilis* apoenzyme forms of LdcB presented in the Hoyland et al. (2014) study possess residual (unmodelled) electron density in the active site, in tandem with modeled co-crystallized D-Ala and phosphate, respectively. There is now detailed information available for both substrate-mimic complex and co-crystallized ligands for LdcB and also a phosphinate transition-state analog for the related LAS member VanXY (Figure 1B; Meziane-Cherif et al., 2014). These observations allow for testable hypotheses regarding the precise nature of the LdcB catalytic mechanism, which is likely to involve Zn-activation of bound water situated adjacently to the scissile bond (Hoyland et al. liken this to that used by the well-documented enzyme pancreatic carboxypeptidase A). Peptidoglycan-metabolizing enzymes are often found in multiprotein complexes; it will be of further interest to observe what precisely regulates LdcB activity, because, in recent years, it has become apparent that the fine structure of the sacculus itself can also influence the function of the proteins that build and tailor it. It remains unclear how tailoring activity perturbs cell shape, although this is a beginning to be filled to some degree by the nascent technique of peptidoglycan imaging using fluorescent D-amino acids (Kuru et al., 2012). Much life left in the "old bag" indeed!

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